Am. J. Hum. Genet. 65:1–6, 1999

NEUROGENETICS '99
Genetics of Angelman Syndrome

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The last 2–3 years have seen a series of exciting developments in the understanding of Angelman syndrome (AS), beginning with identification of the disease gene as a ubiquitin ligase gene (the first single-gene disorder of the ubiquitination pathway) and the demonstration of brain-specific imprinting for this gene. The imprinting center (IC) that regulates expression of genes in this region has been delineated, and a mouse model of the genetic defect has been characterized in detail, providing the first evidence for a mammalian gene that is required for long-term potentiation (LTP). AS is a neurologic disorder characterized by severe mental retardation, usually with the failure to learn speech; seizures that are accompanied by electroencephalogram abnormalities; a movement disorder, usually including gait ataxia and/or tremor of the hands; unusual behavior, including happy demeanor, frequent laughter, and easy excitability; delayed head growth with microcephaly by age 2 years; and sleep disturbance (Williams et al. 1995). A summary of earlier observations with detailed bibliography (Ledbetter and Ballabio 1995) and more-recent reviews of the molecular basis of AS are available (Lalande 1996; Jiang et al. 1998*b*; Nicholls et al. 1998).

Figure 1 depicts significant features of the AS and Prader-Willi syndrome (PWS) region of human chromosome 15q11-q13. A common interstitial ~4-Mb deletion in this region is found in both AS and PWS, but the phenotypes in the two conditions are quite distinct, as is their pattern of inheritance: deletions on the paternal chromosome cause PWS, whereas those on the maternal chromosome cause AS. Consistent with this unusual parental effect, many but not all of the genes and transcripts from this region are subject to genomic imprinting. Thus, the *SNRPN* gene, encoding small nuclear ribonucleoprotein polypeptide N, is paternally expressed and maternally silenced in all tissues examined and represents an important landmark within the region. The *SNRPN* promoter is found within a CpG island that is completely methylated on the maternal chromosome and completely unmethylated on the paternal chromosome. A bipartite imprinting center overlaps this promoter; small deletions of the IC are implicated in imprinting defects that can lead to AS or PWS. Other notable loci in this region include the paternally expressed *IPW, ZNF127,* and *NECDIN* genes; the imprinting of *UBE3A* is tissue-specific, with maternal deficiency causing AS, and the imprinting status is uncertain for a cluster of $GABA_A$ receptor genes. The albinism locus (*P*) is not subject to genomic imprinting; the *HECR2* gene, which encodes a highly conserved giant protein, and *MN7* map near the common PWS/AS deletion breakpoints.

AS is estimated to occur in 1/15,000 births, and the genetic basis for the disorder is unusually complex (table 1). A great majority of patients (65%–75%), designated type Ia in table 1, carry de novo interstitial deletions of maternal chromosome 15q11-q13. These deletions are thought to occur through unequal crossing over between complete or truncated copies of the *HERC2* gene, which form a set of low-copy repeats (Ji et al. 1999). The common deletion is readily diagnosed by use of FISH, and its parental origin can be determined by methylation analysis; in maternal deletions, characteristic of AS of this type, the differentially methylated CpG island encompassing the promoter for *SNRPN* shows only the paternal, unmethylated pattern. Recurrence risk for this group of patients is extremely low. Closely related to this common deletion is a group of very rare mutations (<1%) including unbalanced translocations or inherited interstitial deletions (type Ib in table 1). For example, a Japanese family with an interstitial deletion that caused AS when inherited maternally, but that yielded a normal phenotype with no signs of Prader-Willi syndrome (PWS) when inherited paternally, was extremely valuable in distinguishing the critical regions for AS and PWS.

Approximately 3%–5% of patients with AS have paternal uniparental disomy (UPD) with maternal defi-

Received April 12, 1999; accepted for publication May 7, 1999; electronically published June 7, 1999.

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This article is dedicated to the memory of our deceased colleague, Claudia Benton, who was an enthusiastic physician devoted to investigation and care of Angelman families.

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0002-9297/99/6501-0002$02.00

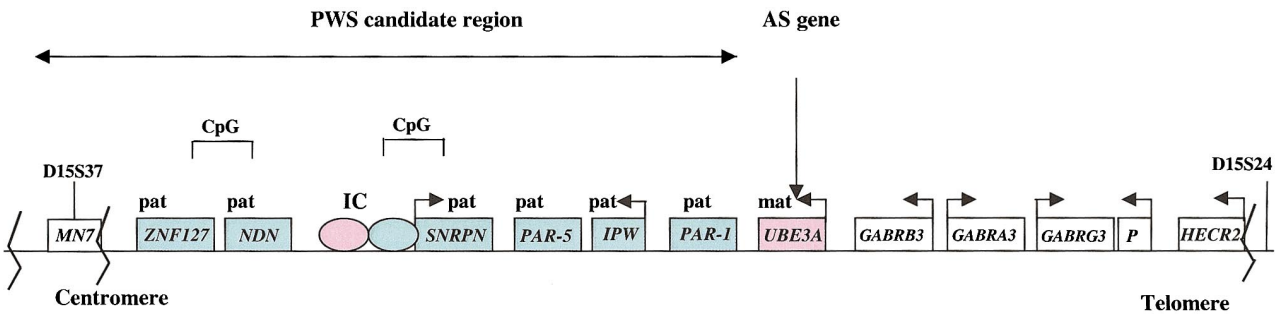

Figure 1 The AS/PWS region of chromosome 15q11-q13 is depicted with various loci and expressed sequence tags indicated. The common telomeric breakpoint and two centromeric breakpoints are depicted as vertical jagged lines and are separated by ~4 Mb; distances are not to scale. Most of the genes depicted also occur as a homologous region on mouse chromosome 7C, and the imprinted expression is generally similar for mouse and human loci, where information is available. The IC is bipartite with the *cis* element of the IC required for maternal-to-paternal switching (deletions in this region cause PWS) depicted as a blue oval overlapping the *SNRPN* promoter; the more upstream element of the IC required for switching from paternal-to-maternal (deletions in this region cause AS) is depicted as a pink oval. Genes or transcripts known to be paternally expressed are colored blue and indicated (pat), whereas the tissue-specific imprinting with maternal expression for *UBE3A* is colored pink and indicated (mat). Transcripts that are known not to be imprinted or those for which imprinting is not certain are shown in white.

ciency for 15q11-q13 and normal parental chromosomes (type IIa). Recurrence risk is low for UPD unless a parental translocation (type IIb) predisposes the family to recurrence. Approximately 7%–9% of patients with AS have "imprinting" mutations, in which the maternal chromosome has a paternal pattern of methylation and imprinted gene expression for 15q11-q13. Among the imprinting mutations, about half (type IIIa) are associated with small deletions that affect the more centromeric portion of the bipartite IC near the *SNRPN* promoter and that make the chromosome unable to switch from the paternal to the maternal pattern of methylation and expression; most cases of AS type IIIa are familial (Ohta et al. 1999). Other cases that are identified as imprinting mutations on the basis of methylation analysis are not associated with detectable deletions in the IC (type IIIb). Familial recurrence has not been reported for this group of patients, and the molecular mechanism causing their defective imprinting mutation is unknown (Buiting et al. 1998).

The *UBE3A* gene, which encodes E6-AP ubiquitin-protein ligase (also known as ubiquitin ligase 3A) was mapped to the AS critical region in 1994, but it was not initially considered a strong candidate as an AS gene because it did not appear to be imprinted. Subsequently, however, point mutations in *UBE3A,* mostly truncating mutations, were found in a significant but relatively small fraction (4%–6%), of patients with AS (type IV). Some of these mutations occurred de novo, but many were inherited, and some families in this group include a large number of affected individuals (Malzac et al. 1998; Fang et al. 1999). When large numbers of type IIIa or type IV AS cases occur in an extended family, the pattern of inheritance is distinctive, in that heterozygotes are normal if the mutant chromosome is inherited from the father but are affected if it is inherited from the mother. The patients with point mutations in *UBE3A* encoding E6-AP have relatively typical and complete AS phenotypes (see Modifier Effects section, below).

A final group of patients (10%–14%) with a clinical diagnosis of AS have no identifiable molecular abnormality (type V). This group of patients likely represents some combination of (1) as yet undetected lesions in chromosome 15q11-q13 that affect the *UBE3A* locus; (2) other novel genetic lesions that map elsewhere but affect expression of *UBE3A;* and (3) incorrect diagnoses, representing genocopies or phenocopies that do not perturb the expression of *UBE3A*. Because studies of patients with AS with normal methylation patterns have identified *UBE3A* mutations much more often in multiplex families than in isolated cases, it is likely that many patients with type V are erroneously diagnosed with AS or that there is yet another molecular mechanism with low recurrence risk to be identified. In most cases, AS can be diagnosed and patients can be counseled adequately by use of a combination of FISH, methylation analysis, and DNA marker studies for UPD, but it is labor intensive to search thoroughly for IC deletions with imprinting mutations or for mutations in *UBE3A*. Because only a small fraction of patients with a normal methylation pattern have identifiable *UBE3A* mutations, the frequency of patients with all laboratory studies yielding normal results (type V) is significant, leaving clinicians and families with uncertain diagnosis and recurrence risk.

Modifier Effects

The *P* locus is not imprinted, but it falls within the ~4-Mb common deletion region and modifies the phe-

Table 1

Angelman Syndrome

Type	Mechanism	Proportion	Methylation[a]	Recurrence
Ia	~4-Mb interstitial maternal del15q11-q13	65%–75%	Abnormal	Extremely low
Ib	Unbalanced translocation or inherited interstitial deletion	<1%	Normal or abnormal	Significant
IIa	UPD maternal deficiency with normal parental chromosomes	3%–5%	Abnormal	Extremely low
IIb	UPD with predisposing parental translocation	<1%	Abnormal	Significant
IIIa	Imprinting mutation with deletion of IC	3%–5%	Abnormal	Significant
IIIb	Imprinting mutation without detectable deletion of IC	3%–5%	Abnormal	Low
IV	Point mutations in *UBE3A*	4%–6%	Normal	Significant
V	AS phenotype with no identifiable molecular abnormality	10%–14%	Normal	Occurs rarely

[a] "Abnormal" indicates that only the paternal, unmethylated pattern is seen on analysis of the *SNRPN* promoter region.

notype to include mild hypopigmentation in the type Ia deletion form of AS. In humans or mice with loss-of-function mutations on both alleles for the *P* or *p* loci, respectively, the phenotype is albinism. The heterozygote phenotype is more obvious in mice but is associated with a mild decrease in pigmentation in humans. Hypopigmentation is not thought to be part of the phenotype observed in type II, III, or IV AS, involving UPD, imprinting mutations, or point mutations in *UBE3A,* respectively.

Most type IV patients with AS have epilepsy, but some reports suggest that epilepsy is more severe in patients with type Ia who carry the ~4-Mb deletion. The difference may be related to the presence of a cluster of $GABA_A$ receptor genes located between *UBE3A* and the *P* locus, as suggested by the finding that a knockout mutation for one of these receptors (*Gabrb3*) causes epilepsy in mice (Delorey et al. 1998). The status of genomic imprinting regarding the $GABA_A$ cluster of receptors is uncertain (see bibliography in Jiang et al. 1998*b*), but maternal deficiency for *GABRB3* may function as a modifier of the epilepsy phenotype, accounting for the possibly greater severity in the deletion cases. Thus, type IV AS is a single-gene disorder with point mutations in *UBE3A,* but type Ia AS represents a contiguous gene syndrome, in which *UBE3A, P,* and perhaps *GABRB3* all contribute to the phenotype. Despite the complexity of AS inheritance, the major phenotypic effects derive in all cases from deficient expression or function of the maternal *UBE3A* allele.

Brain-Specific Imprinting of *UBE3A*

Although imprinted expression was not found for *UBE3A* in cultured human cells, once mutations causing AS were found, it was quickly discovered that *UBE3A* is imprinted in human brain and that the paternal allele is silenced in that organ (Rougeulle et al. 1997; Vu and Hoffman 1997). More-detailed in situ hybridization data from the mouse indicates that *Ube3a* is preferentially expressed from the maternal allele with silencing of the paternal allele in Purkinje cells, hippocampal neurons, and olfactory mitral cells, whereas expression is not imprinted in most parts of the brain or in other somatic tissues. This tissue specificity was first described in UPD mice (Albrecht et al. 1997) and has been confirmed by use of a gene-targeting mutation (as described below; also see Jiang et al. 1998*a*). The maternal deficiency of *Ube3a* in Purkinje cells in mice could account for the ataxia and tremor seen in patients with AS, and the deficiency in the hippocampal neurons may explain learning deficits and epilepsy, but the sublocalization of tissue-specific imprinting in the brain has yet to be determined in humans. As discussed below, indirect evidence, based on excessive cytoplasmic p53 in the AS brain, suggests that the gene is imprinted in human Purkinje cells.

The molecular basis for the tissue-specific imprinting is unknown but may be similar to the mechanisms used at other imprinted loci or for X inactivation. *UBE3A* uses multiple promoters and is subject to complex alternative splicing of 5′ untranslated exons, and there is precedent for alternative promoters involved in tissue-specific imprinting of *IGF2* (Vu and Hoffman 1994). Molecular lesions that affect control elements separated from the structural gene by as much as a megabase are known to cause AS. One *cis* element that has been mapped to a 1.15-kb region in the more centromeric portion of the bipartite IC (Ohta et al. 1999) is required for switching from paternal to maternal epigenotype. There could well be tissue-specific enhancers or locus control regions (Tanimoto et al. 1999) between the IC and *UBE3A* or even flanking either of these sites. Also potentially relevant to the mechanism of imprinting is the report of an antisense transcript for the 3′ untranslated region of *UBE3A* (Rougeulle et al. 1998). Although molecular lesions involving other putative *cis*-acting elements may occur, it is the paternal silencing of *UBE3A* that is tissue specific. Activation of maternal expression is not involved, because the maternal allele is expressed quite ubiquitously.

The Biochemistry of E6-AP, Encoded by *UBE3A*

The E6-AP protein, the product of the *UBE3A* gene, was initially identified because of its ability to interact

with the E6 protein of human papillomavirus to promote the ubiquitination and degradation of p53 (Huibregtse et al. 1991). Ubiquitination (reviewed in Hershko and Ciechanover 1998) involves four different classes of proteins that act together to target selected proteins for degradation. An E1 enzyme begins the process by forming a high-energy thioester bond between a cysteine of its active site and the C-terminal amino acid of ubiquitin. Activated ubiquitin is then transferred to a series of E2 enzymes that also form thioester-linked complexes with ubiquitin. Ubiquitin is then covalently attached to a protein substrate directly from an E2 enzyme or is transferred to a ubiquitin protein ligase (E3), which, in turn, ubiquitinates the target protein. E3 proteins, including E6-AP (Scheffner et al. 1993), provide specific recognition of substrate proteins. E6-AP is the founding member of the homologous to E6-AP C-terminal (hect) domain family of E3 proteins that now includes as many as 20 different hect family proteins. Three other families of E3 ligases have been described: E3 proteins designated anaphase-promoting complex (APC) are implicated in control of the cell cycle, as are members of the phosphoprotein-ubiquitin ligase family, referred to as the Skp1-cullin-F box-protein (SCF) family. Another group of E3s, the N-end rule family, recognize substrates based on a characteristic N-terminal sequence (Hershko and Ciechanover 1998). A final class of ubiquitination factor, termed "E4" by Koegl et al. (1999), was recently shown to allow target proteins to progress from the oligo-ubiquitinated state, which occurs by the action of hect domain-type E3 ligases, to multiubiquitination and proteosomal degradation.

E6-AP can interact with and, presumably, accept ubiquitin from several E2 enzymes, including UbcH5, UbcH6, UbcH7, and UbcH8. Subsequently, E6-AP ubiquitinates at least four proteins, but its targets might easily number in the dozens or hundreds. In addition to p53, the targets include HHR23A, a protein homologous to the yeast DNA repair factor RAD23; MCM-7, a protein implicated in chromosomal replication; and E6-AP itself. In addition to its ubiquitination activity, E6-AP can also serve as a transcriptional coactivator for steroid hormone receptors (Nawaz et al. 1999). Transcriptional regulation and ubiquitination appear to be independent activities, because an N-terminal domain appears to mediate the former activity, whereas the ubiquitination domain is located at the C-terminal portion of the protein.

Mouse Models of Angelman Syndrome

Mice with paternal UPD for chromosome 7 were first reported as a model for AS (Cattanach et al. 1997), although a previously known radiation-induced deletion (*p30Ub;* Johnson et al. 1995) that lacks both the *p* locus and *Ube3a* could also be used to produce maternal deficiency for *Ube3a*. Both of these models involve large regions of mouse chromosome 7C and could affect multiple loci. We produced a null mutation in *Ube3a* by using gene targeting and found that maternal deficiency mice show no detectable expression of the locus in hippocampal neurons or in cerebellar Purkinje cells (Jiang et al. 1998*a*). The maternal deficiency (AS) mice demonstrate motor dysfunction, inducible seizures, and a defect in contextual learning and hippocampal LTP. Contextual fear conditioning is an associative learning behavior implicated in rat brain–lesion studies to involve hippocampal function. LTP is an electrophysiological phenomenon whereby stimulation of presynaptic axons increases the strength of connections to postsynaptic neurons for days to weeks and is widely regarded as a form of neuronal plasticity that is relevant to learning and memory. LTP is generally considered the strongest candidate cellular mechanism for learning and memory (see Meiri et al. 1998 for a dissenting view; Stevens 1998). The defect in LTP, which is quite prominent in AS mice, represents the first evidence for a role of ubiquitination in mammalian LTP. In *Aplysia,* a much-studied model system for the molecular basis of associative learning, ubiquitin-dependent proteolysis is implicated in the synaptic changes that occur in long-term facilitation. Nevertheless, it is uncertain whether E6-AP plays a direct or indirect role in affecting LTP. Because the mice have normal neuroanatomy and normal baseline synaptic transmission (Jiang et al. 1998*a*), the defect in LTP may represent a primary abnormality. AS mice have an increased abundance of cytoplasmic p53 in Purkinje cells and some hippocampal neurons, which suggests that E6-AP regulates the abundance of p53 directly in vivo through ubiquitination.

Pathogenesis of Angelman Syndrome

Despite the flood of recent data on *UBE3A* expression and E6-AP biochemistry, the mechanisms whereby maternal deficiency of E6-AP causes the phenotypic features of AS remain largely unknown. Significant Purkinje cell and granule cell loss and increased abundance of p53 in Purkinje cells were found at autopsy in a 21-year-old patient with a clinical diagnosis of AS (Jay et al. 1991; Jiang et al. 1998*a*). However, normal neuropathology has been reported in a 3-year-old patient (Kyriakidas et al. 1992), which suggests that Purkinje cell death is, at most, a late effect of maternal deficiency for *UBE3A*. Moreover, the neuroanatomy in AS mice up to 3–4 mo of age is also normal, despite their phenotypic abnormalities. The elevated cytoplasmic levels of p53 in human Purkinje cells in AS suggest that expression of *UBE3A* is imprinted in this cell type in humans, as it is in mice. This high level of p53 might initiate apoptosis and contribute to cell loss at later ages. Candidate proteins in Purkinje cells and proteins implicated in LTP in

the hippocampus can be evaluated as potential targets for ubiquitination by E6-AP. *Ube3a* mutant mice should provide an additional means to identify target proteins that are found in Purkinje cells or hippocampal neurons at elevated levels relative to wild type. Finally, the transcriptional coactivation capacity of E6-AP must be considered, along with ubiquitination, as possibly contributing to the pathogenesis. However, the finding that AS can arise from missense mutations in the ubiquitination domain may argue that ubiquitination defects per se are sufficient to cause the disease. The transcriptional function of some of these mutant E6-AP proteins appears to be preserved in tissue culture studies (Nawaz et al. 1999).

Potential for Additional Disorders of Ubiquitination and LTP

AS represents the first clear example of a single-gene disorder involving the ubiquitination pathway in humans, although numerous such defects have been described in yeast and *Drosophila* (see Jiang et al. 1998*a* and references therein). In mice, the disruption of an E2 locus, *UbcM4,* leads to embryonic lethality (Harbers et al. 1996), and a mutation in an E3 ubiquitin-protein ligase, encoded by the *Itch* locus, causes a complex spectrum of immunologic and inflammatory abnormalities (Perry et al. 1998). The velocardiofacial/DiGeorge syndrome may be caused by a defect in a protein involved in degradation of ubiquitinated proteins (Yamagishi et al. 1999). Given the large number of E2 and E3 loci in mammals, it is likely that additional single-gene disorders involving the ubiquitination pathway will be identified in humans. In addition, abnormalities of ubiquitination are likely to be secondarily involved in many pathological conditions, and they are already implicated in neurodegenerative triple-repeat disorders (Cummings et al. 1998; Chai et al. 1999), Alzheimer disease, and other neurodegenerative disorders (Alves-Rodrigues et al. 1998).

There is little precedent for the effects of abnormalities of LTP on human learning and behavior. Since mice with AS have an abnormality of LTP, it is of particular interest to carefully examine the cognitive features of human patients with AS. It is likely that additional instances will arise in which mice and humans carry mutations in orthologous genes and a defect in LTP will be shown in the mouse. Such models hold the promise of a deeper understanding of the relationships between the cellular events of LTP and the behavioral changes we observe as learning and memory in mice and humans.

Acknowledgments

We apologize to the many authors whose primary data could not be cited because of limitations of space. We thank Grace Watson for great assistance in preparation of the manuscript. This work is supported by NIH grant HD 37283.

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